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TECHNICAL REPORT

TR 76-44 FSL

SERIES: MICROBIOLOGICAL DETERIORATION NO. 14

PREVENTION OF FOOD RESIDUE SPOILAGE IN WASTE PACKAGING FROM FEEDING SYSTEMS UTILIZED BY PERSONNEL OPERATING IN A CLOSED GASEOUS ENVIRONMENT - REQUIREMENT NO. AF 4-12

FINAL REPORT

PROJECT 1Y762724AH99 TECH AREA AH99BD COVERING

THE PERIOD 26 SEPT. 1973 - 26 SEPT. 1975

November 1975

Approved for public release;
distribution unlimited.

UNITED STATES ARMY
NATICK RESEARCH AND DEVELOPMENT COMMAND
NATICK, MASSACHUSETTS 01760

Food Sciences Laboratory

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Under certain military requirements, personnel are required to live for extended periods in closed gaseous environments. Food wastes and trash produced under these environments can not be disposed of in the usual manner. It was determined that limited quantities of food waste and trash may be compacted and preserved against gas and odor production for up to 30 days storage at room temperature. Several disinfectants were evaluated as preservatives. The addition of salt to the food wastes and trash prior to compaction proved to be simple, inexpensive and an effective way of preventing odor and gas production of the compacted

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waste during a 30 day incubation period under laboratory test conditions. The procedure requires field testing prior to final adoption by the Air Force.

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REQUIREMENT NO. AF 4–12

INTRODUCTION

Stabilization of food waste until it can be disposed of is a problem for both military and civilian organizations. Although final disposal of food waste is usually brought about through natural decomposition or decay of organic matter common to food waste or by incineration, there are occasions when prompt disposal is not possible and food wastes generating obnoxious odors and gases must be held for some period of time until disposal is possible.

Because of military necessity, personnel are frequently assigned to live and perform special assignments in closed environments where the normal routes for the prompt disposal of food wastes are denied. Crews assigned to space craft flights of long duration or to extended alert duty in missile sites are in such situations and must live in close proximity to their garbage and trash. Refuse remaining from partially eaten meals and from food prepration require some type of treatment to render it inocuous during storage within the confines of the space craft vehicle or missile site. Without proper management and stabilization, disagreeable and hazardous metabolic products evolving from the microbial attack and putrefaction of the food wastes stored at room temperature could subject the crews to toxicological risks which could seriously endanger their ability to carry out their duties in an already prevailing stressed environment.

Previous studies involving the control of waste putrefaction in space flight have been reported by Roth et al.[1] Possible control and preservation procedures discussed include jettisoning, heating, refrigeration, desiccating and treating with antimicrobic chemicals. Under an AF contract between the Aerospace Medical Laboratory, Wright-Patterson Air Force Base, Ohio and the Whirlpool Corp., St. Joseph, Mich., studies based on recommendations from this Laboratory[2] showed that 2% of Disinfectant, Germicidal and Fungicidal, Phenolic (NSN 6840-985-7118) was sufficient to effect near sterilization

[1] Roth, N.G., R.B. Wheaton and H.M. Morris. 1962. Control of Waste Putrefaction in Space Flight. Dev. Ind. Microbiol. 3: 35-44.

[2] Mizuno, W.G., M.R. Rogers and A.M. Kaplan. December 1958. Development of a General Purpose Disinfectant for Military Use. Soap and Chemical Specialities. Part II, 105-111.

(99.999% reduction) of the bacterial cells in a mixed food waste. A 5% concentration of this disinfectant was also used to disinfect fecal wastes aboard the Gemini and Apollo space craft. Bourland et al.[3] in 1971 reported the relative effectiveness of 8-hydroxyquinoline sulfate (8-HQS) and alkyl dimethyl benzyl ammonium chloride (ADBAC) in a series of laboratory tests designed to control the growth of microorganisms in aerospace food waste. A control of 1% 8-HQS and 2% ADBAC maintained microstatic control in certain food items. It was noted, however, that "neither compound was tested in the presence of food and coliforms or yeast and mold because these microorganisms were not detected in the control samples". The authors also recommended that sodium chloride be evaluated as a means of waste stabilization since it can bind the essential water required by the putrefying organisms.

In FY 1974, a project was initiated by the Applied Microbiology Group, Pioneering Research Laboratory, U.S. Army Natick Laboratories (renamed U.S. Army Natick Research and Development and Command – NARADCOM) to service a new requirement designated as AF 4--12 under the DoD Food RDT&E Program. This requirement was aimed at developing techniques for preventing the spoilage of food residues in used packaging feeding systems utilized by personnel confined in a closed gaseous environment. Specifically, the AF requires contingency plans for several weapon systems involving the extension of nominal 12 hour missions to missions lasting 72 hours or longer. Food residues from the feeding of personnel aboard aircraft involved in a 12 hour mission present no hazard, but during longer missions the food residues from meals eaten by the crews aboard these aircraft can decompose and give off noxious odors and/or toxic gases which may present a morale and potential health and safety hazard. Similarly, any food waste control system developed and designed for aircraft and/or spacecraft could also be applicable to missile sites when operating in the ready state where crews live in a semi-enclosed environment for up to 30 days.

[3]Bourland, C.T., C.S. Huber and N.D. Heidelbaugh. 1971. Relative Effectiveness of 8-Hydroxyquinoline Sulfate and Alkyl Dimethy Benzyl Ammonium Chloride in the Stabilization of Aerospace Food Waste. J. Milk Food Technology. **34**: 478-481.

AIR FORCE GUIDE LINES

Guidelines issued by the AF under the 4–12 requirement include the following essential characteristics:

a. Products or technology developed will prevent the growth of microorganisms in most food residues.

b. The products developed will not be toxic to humans or present a health hazard during use.

c. The desired products shall have a useful storage life of at least five years.

d. The desired products shall not impart a noxious odor when appropriately used.

e. The desired product shall be capable of use with polyethylene containers and be non-corrosive to stainless steel and aluminum.

f. In order to handle the volume of trash generated, the food and packaging waste must be compacted using a Kitchen Aid Compactor, Model KCS-100 which is to be retrofitted into specified aircraft. The compactor and the KCB-1 3.0 mil polyethylene bags used to hold the compacted waste are manufactured by the Hobart Manufacturing Co., Troy, Ohio.

g. Any automatic disinfectant dispensing device required must be designed to fit within the space occupied by the compactor specified.

MATERIALS AND METHODS

Source and Type of Food Waste/Trash

In the early phase of this investigation[4] most of the food waste/trash was obtained from the NARADCOM Experimental Kitchen of the Food Engineering Laboratory. The trash was typically a mixture of paper, aluminum flexible trays, food scraps including liquids such as soup and beverages and material that would presumably exemplify the wastes that the compactor would be expected to handle under everyday use in its intended

[4] Rogers, M.R. and A.M. Kaplan. 1975. Prevention of Spoilage of Food Residues in Used Packaging from Feeding Systems Utilized in a Closed Gaseous Environment. Memorandum Report No. 1, Project 1J662713A034, U.S. Army Natick Development Center, Natick, MA, 14 Feb. 1975.

environment. The bags of trash provided by the kitchen were stored in the cold room at −15 C until used or for no longer than three days. In some of the tests (bags #67 to 111), a uniform garbage consisting of mashed potatoes, meat and vegetable were mixed with paper wastes, napkins and small cardboard boxes. In the final phase of this study, the food waste consisted of frozen precooked meals typical of the meals consumed by AF personnel aboard aircraft. The meals used in these tests (bag #129 to 145) included a breakfast menu (NSN 8970-165-6904, Ham with Eggs) a luncheon menu (NSN 8970-165-7907, Salisbury Steak) and a dinner menu (NSN 8970-165-6902, Beef Sirloin) all of which were produced by J & B Food Distributors, Pemberton, N.J. 08068.

Compaction Procedure

The Kitchen Aid Compactor and polyethylene bags used in all experiments were purchased from the Hobart Manufacturing Co. as previously specified. Three compactions of trash were normally run for each bag (simulating the food/trash residue from three meals/day) which usually yielded between fifteen and twenty-five pounds of waste. It was necessary to place sharp objects such as plastic spoons in the center of the trash to be compacted to prevent them from ripping the polyethylene bag. Metal cans and glass jars were also placed in the center of the trash with most of the paper waste placed around them. If much liquid was to be added such as one or more quarts of disinfectant and/or soup, additional paper towels were also placed in the bottom of the bag to soak up the excess liquid. A second polyethylene bag was also used to overwrap the bag of compacted trash thus providing double protection against leakage since liquid would often seep through the bottom seams of the compacted bags where the layers of plastic join to form a heat seal corner. It was necessary, therefore, to reheat seal each bag prior to use in order to guarantee tightly sealed bags for each test in which liquid disinfectant was to be added. When solid inhibitors were used (salt, Hyamine and Aquacide III) the bags were not reheat-sealed but tied off with a tie or string since free liquid was held to a minimum of wasted coffee, tea, milk, etc. which was readily soaked up by the paper trash, thus preventing the bags from leaking. Small rips and holes were commonly seen in the polyethylene bag after removal from the compactor. To provide an air-tight environment for the trash, the compacted bag was tied off with a tie or piece of string, placed in a second bag, which was in turn either heat sealed or tied off with a wire tie. The heat-sealer used was a Vertrod Impulse Heat Sealer,* Model 24PR/OB and settings used that appeared to provide the best heat seals were: Heat-5, Dwell-5 and Recycle-0.

Testing Integrity of Heat Sealed Plastic Bags

The quality of the heat seals were first checked by sealing a piece of dry ice within a small (approximately 6" x 4") hand-made plastic bags fabricated from KCB-1

*A product of Vertrod Corp., Brooklyn, N.Y. 11234.

polyethylene bags. As the ice sublimed and filled the bags with gaseous CO_2, the bags were submerged in water to observe for leaks indicated by the evolution of CO_2 bubbles. The laboratory heat seals almost invariably remained leak free as the bags expanded. However, when the original heat seal found across the bottom of each KCB-1 bag was tested, it almost always was the source of a leak. This was the reason an additional heat seal was applied across the bottom of each KCB-1 bag before use.

In order to determine the amount of CO_2 pressure the double sealed KCB-1 bags could withstand, pressure measurements were taken with an oil U-tube manometer equipped with a rubber tube attached to a hypodermic needle. The needle was inserted through a globule of a Raycohesive B-2 Fast Drying Adhesive* previously applied to the KCB-1 bag and allowed to dry in order to prevent gas leakage during the sampling procedure. The readings taken were the differences in millimeters of the two columns of oil (h) at the time that they were furthest apart. At this point the pressure produced by the sublimed dry ice would be at the limit that the bag could tolerate (0.13 psi, 0.0009 Pa). The columns of oil would quickly return to the same level when a leak in the bag developed. Using the proper conversion factors the pressure in mm oil could be converted to psi and then to the SI unit Pa.

Initial Compaction Experiments Without Disinfectant

After each bag of compacted trash was heat sealed, it was labeled with a number and the date, and an initial measurement of bag circumference taken perpendicular to the heat-seal. No disinfectant was added to the first series of bags (#1 to 24) as it was necessary to determine if ballooning could indeed be observed and used as a criterion on which to base effectiveness of the disinfectant. The bags were incubated to accelerate microbial growth and gas production which would, in turn, cause ballooning of the sealed KCB-1 bags. The first 18 bags were stored in Tropical Chamber DB019 at a temperature of 33 C and a relative humidity of 96%. Measurements of circumference were taken daily to quantify any ballooning that may have been observed.

Odor within the trash bags was also used as a rating criterion for effectiveness of disinfectant activity. On the day of the final measurement for each bag, the bag would be cut open and the contents checked for odor by smelling. The relative values assigned to each bag were as follows: 0=no odor, =very slight odor; +=some odor, slightly disagreeable; ++=strong odor, disagreeable and usually acrid; +++=acrid odor and very disagreeable.

*Raycon Instrument Co., Boulder, CO.

Compaction Experiments with Disinfectant and Other Inhibitors

Disinfectant, Germicidal and Fungicidal, Concentrate (Phenolic, Dry-Type), as stated previously, has maintained an excellent record of performance as a fecal disinfectant for the astronauts assigned to long-term space missions as demonstrated in the Gemini and Apollo flights. Because of this success record, it was considered to be a good candidate disinfectant for these studies since it met for the most part the original criteria specified by the AF. Dr. John Vanderveen, USAFSAM-VNAN, Brooks AFB, Texas 78235 originally questioned its use because of some preliminary studies conducted at Brooks Air Force Base which indicated that the disinfectant might leave a residual of phenol in the air that could present a toxicological hazard. However, since no additional toxicity data were developed to verify this concern, verbal approval to use this disinfectant in these studies was received since our preliminary data revealed that the three phenolic salts (26% sodium-ortho-phenylphenate, 37% sodium 4-chloro-2-phenylphenate, 12% sodium 6-chloro-2-phenylphenate and 9.0% sodium para-phenylphenate) comprising the disinfectant have no vapor pressure in the pure state. However, because the disinfectant is compounded with technical grade and not pure grade compounds, they do undergo some slight hydrolysis in solution and a faint phenolic odor can be detected. This phenolic odor, however, is markedly reduced by dissolving the disinfectant in propylene glycol as was done aboard the Gemini and Apollo flight craft. Since the disinfectant is a powder, it can also be made non-dusting by packaging it in a water soluble pouch made from polyvinyl alcohol. With this background information, it was decided to evaluate the disinfectant as a possible food waste disinfectant.

The phenolic disinfectant was prepared for testing by dissolving it in the necessary volumes of water to yield 14,000 mg/l to 5.0% solutions. One half liter of the disinfectant solution at the concentration under test was added to each bag and distributed throughout the waste by pouring it over the waste before compaction. In adding the disinfectant an attempt was made to saturate as much of the waste as possible to be sure that all of it came in contact with the disinfectant. When soup was to be added to the trash, a quantity of disinfectant was added to the soup. In several experiments, the food waste was also soaked in the disinfectant (bags #52, 54 to 56, 58, 59, 61, and 63 to 65) prior to mixing with the trash and compaction.

Sodium Chloride

Salt had been suggested by Bourland et al (reference 3) as a possible means of stabilizing aerospace food waste. Hof[5] found that most species of bacteria tested in salt brines did not tolerate more than 6% salt. Yesair and Cameron[6] showed that 2% sodium

[5] Hof, T. 1939. Investigations Concerning Bacterial Life in Strong Brines. Rec. Trav. Bot. Neerland. **32**: 92-173.

[6] Yesair, J. and E. J. Cameron. 1940. Studies in Processing Canned Meats. The Canner. **90**: 78.

chloride brought about a 70% reduction of *Cl. botulinum* No. 62A spores, while 3.69% sodium chloride was required for over 99% reduction. In an attempt to determine what level of salt would control microbial spoilage of compacted food waste, salt was evaluated at 2 to 10% in several experiments. One pound 10 oz. (26 oz. = 737 g) boxes of common non-iodized granulated table salt were used throughout the tests.

A series of fermentation, water-displacement experiments were first used to screen the various concentrations of salt required to control gas production. Figure 1 shows the equipment used in these experiments. The system essentially involved the use of Berzelius, tall form, graduated beakers without spout to which was added 300 ml of either mashed potatoes, potatoes with 1% glucose or approximately 600 grams of a uniform garbage consisting of potatoes, ground raw beef, mixed vegetables and cup-cake like dessert. The garbage was homogenized in a Waring blender with salt added to give concentrations of approximately 2, 4, and 6%. The beaker was stoppered with a rubber stopper through which a glass tube was inserted to permit any gas evolved from the fermenting garbage to escape and to displace water in the inverted volumetric cylinders. The system was incubated at room temperature.

One additional simple screening system devised consisted of 250 ml graduated cylinders to which was added approximately 130 ml of mashed potatoes containing various levels of salt. The cylinders were also incubated at room temperature, covered with glassine paper and allowed to ferment. Figure 2 shows the physical set-up of this method. In the samples containing 0 and 1% salt, the gas production due to microbial fermentation caused the potatoes to expand from the initial filling to 140 to 150 ml mark to $>$ 250 ml for the control and to approximately 180 ml for the 1% salt. The 2, 4, and 6% salt showed no change from the initial filling of the cylinders, thus indicating no microbial attack. Although this proved to be a simple screening test the data developed from it was not very reproducible and its use was therefore discontinued.

The first series of compaction experiments utilized 2% salt, based on the effective levels obtained with the screening experiments. A mixed garbage, including paper plates, cups, towels, as obtained from the experimental kitchen was used in these tests. The total bag contents for each bag weighed between 5 and 10 lbs (2268 to 4536 g) with the amount of salt added adjusted to give 2% salt per bag. The salt is applied to the trash by first placing a layer of paper towels or napkins on the bottom of the bag and sprinkling with a thin layer of salt. A layer of garbage is next added often mixed with cups, aluminum trays or other disposable items such as plastic knives, spoons and forks. Any eating utensil such as plastic or paper cups, cans, bottles, trays etc was first sprinkled with salt on the inner surface and then dispersed into the compactor, open side facing up so that any liquids would tend to stay within each container. The paper wastes were interspersed with the food wastes, usually ending up with a layer of paper towels on the top of the trash in order to prevent build up of food residues on the compaction

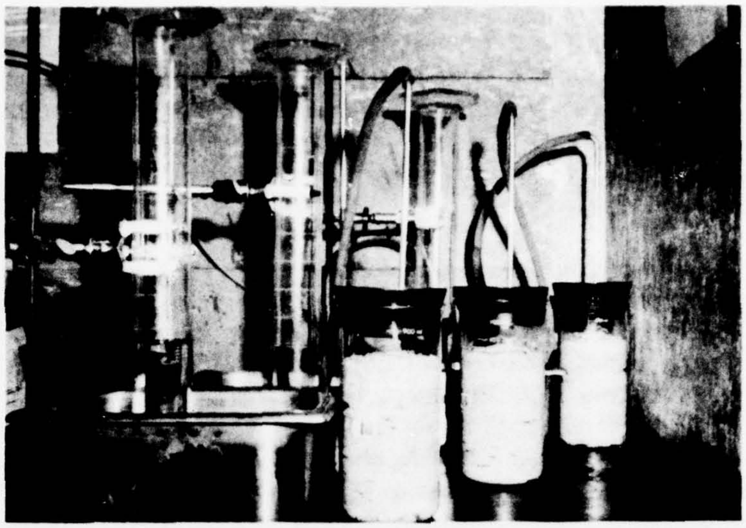

Figure 1. Water displacement tests used to screen the effectiveness of several concentrations of salt added to prevent microbial gas evolution from a uniform garbage.

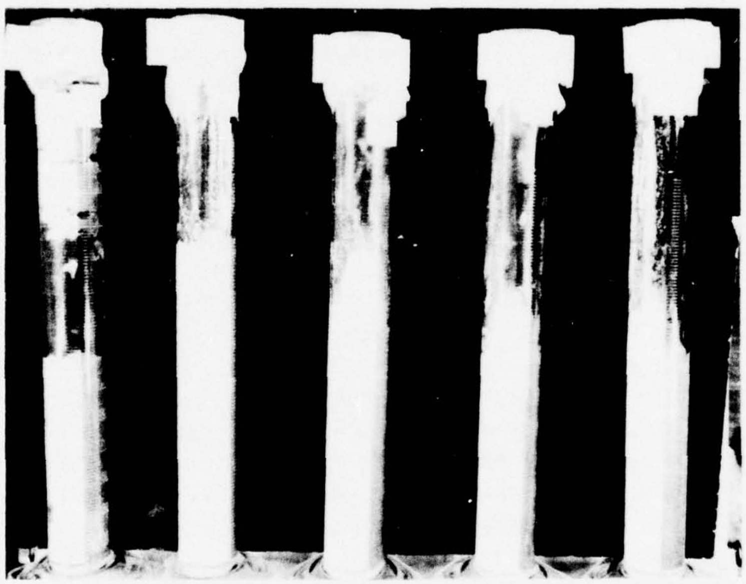

Figure 2. A simple screening procedure used to evaluate the effectiveness of various concentrations of salt to prevent the fermentation of mashed potatoes. The volumetric cylinder on the left (control (0) and the 1% salt) showed little or no control over fermentation and gas production whereas those cylinders containing 2, 4 and 6% salt showed no gas production and therefore indicating these levels of salt prevented microbial attack.

ram. As the layers of waste food residues were added to the compactor, the application of salt was repeated as often as necessary; usually three compaction cycles were completed for each bag before the bag was removed and placed into incubation.

Sodium Silicate

Since sodium silicate has been shown to be biocidal[7] to *Salmonella pullorum* between 2.5 and 5.0% after 30 and 120 minutes contact and because it forms insoluble corrosion products which would tend to make it less corrosive than salt, it was decided to evaluate its inhibitory properties in the fermentor system previously described as well as with the compactor.

Propylene Glycol

Propylene glycol is a nontoxic antifreeze used in the dairy and brewery industries, and is also used as an inhibitor of mold growth and fermentation. Since it also is a humectant and solvent, it appeared to offer some promise as a prospective inhibitor of microbial growth in the compacted food wastes. Unfortunately propylene glycol is a syrup liquid that does not wet out or penetrate all surfaces readily, and is therefore difficult to dispense evenly while pouring over the garbage/trash mixture prior to compaction. Prickett et al[8] found that concentrations of 2 to 5% had no significant antimicrobial effect, while others found 10 to 20% concentrations are required to control growth. Propylene glycol was first screened at 2 and 6% in the fermentors followed by tests in the compaction bags (#114 and 118) at 2 and 6% respectively. It was also tested at 26% in bag #137.

Flaked Polyethyleneglycol

Aquacide III (Calbiochem) is a flaked polyethyleneglycol, and was evaluated for its antimicrobial properties since it would obviate the drawback of a liquid such as propylene glycol which tends to seek any hole or tear in the plastic bag. Aquacide III, however, is very difficult to distribute evenly throughout the waste material. It is a dry flake

[7] McCulloch, E.C. et al. 1943. The Disinfection of Baby Chick Shipping Boxes with Sodium Silicate. Am. Jr. Vet. Res. **4**: 95.

[8] Prickett, P.S., H.L. Murray and N.H. Mercer. 1961. Potentiation of Preservatives (Parabens) in Pharmaceutical Formulations by Low Concentrations of Propylene Glycol. J. Pharm. Sci. **50**: 316-320.

typically used as a dehydrating agent in the drying tubes and dialysis bags. Although Aquacide III is expensive ($1.60/lb), it does overcome the physical disadvantages of liquid propylene glycol. It was evaluated at 8.6 to 9.4% concentrations in bags #143 to 145.

Hyamine 1622

Bourland et al (reference 3) evaluated a a specially prepared 50% active quaternary ammonium compound for sterilizing aerospace food waste because of its many desirable technical characteristics. It is odorless, water soluble, solid in form, non-gas forming, nontoxic and effective in small concentrations. As a possible alternative to the use of salt, a 50% active diisobutylphenoxyethoxyethyl dimethyl benzyl ammonium chloride (Hyamine 1622) was also evaluated in this study for its ability to stabilize the odor and gas production from compacted food waste/trash. Hyamine 1622 (Rohm and Haas Co.) is a liquid preparation, and the powdered product was prepared by absorbing the liquid Hyamine onto sodium sulfate. The powdered Hyamine was prepared by mixing 20 grams of Hyamine 1622 with 100 grams of sodium sulfate. A 50/50 mixture of the resultant product was prepared with ethyl alcohol and oven dried at 50 C. Up to 20% Hyamine can be absorbed onto several salts, but in this instance the final product represented 10% Hyamine 1622. The Hyamine was first evaluated in the small fermentor test system at 6% concentration using a uniform garbage previously described. The Hyamine was also evaluated in bags #138 to 142 at concentrations ranging between 8.6 and 9.5% where the absorbed Hyamine was dispersed throughout the trash in a manner previously described with the salt treatment.

8-Hydroxyquinoline Sulfate (8-HQS)

The literature reports varying degrees of effectiveness of 8-HQS against fungi and bacteria.[9,10] 8-HQS* is a chelating agent and such compounds act not through removing the metal but by forming lethal complexes with it. A paradoxical effect has been demonstrated for 8-HQS since in ordinary broth there is enough iron present to render

*Limited production under a NASA contract with the Whirlpool Corp., Benton Harbor, MI.

[9]Albert, A. 1944. Cationic Chemotherapy with Special Reference to the Acridines. Med. J. Australia, i: 245-248.

[10]Albert, A. et al. 1947. The Influence of Chemical Constitution on Antibacterial Activity, Part III. A Study of the 8-hydroxyquinoline (oxine) and Related Compounds. Brit. J. Exp. Path. **28**: 69-87.

it antibacterial. When the concentration of the compound is increased it results in the reduction of the antibacterial action. In the total absence of iron or copper, 8-HQS exhibited no antibacterial activity at any concentration. Similarly, in distilled water, the compound is noninhibitory against microorganisms. However, Bourland et al (reference 3) showed that a concentration of > 1% of a tableted form of 8-HQS containing magnesium in aerospace food waste provided no growth in the samples tested.

The current price for 8-HQS is $7.10/lb in a minimum order of 100 to 400 lb (453.6 to 1814.4 kg) lots and the only commercially available source is Ashland Chemical, Columbus, OH. Because of the cost and the minimum order requirements, the compound was not purchased and, instead, eight of the 8-HQS tablets of the same lots used by Bourland et al. (reference 3) were obtained through the courtesy of Dr. John Vanderveen, USAF SAM-VNAN, Brooks AF Base, TX 78235. Prior to 1 April 1974, it was recommended that we not evaluate this compound since simulation studies at the USAF School of Aerospace Medicine, Brooks AFB, TX in support of the Man Orbital Laboratory (MOL) program indicated a high incidence of methemoglobin was formed in subjects who consumed prototype space foods. Measurements made of returning astronauts also revealed a high level of circulating methemoglobin. Since it was known that compounds similar in structure to 8-HQS caused the formation of methemoglobin, it was decided to remove the compound from the MOL feeding system. However, it was later learned from Dr. Vanderveen that subsequent studies with human subjects showed a normal methemoglobin and the earlier warning against using this compound was withdrawn. In a limited number of compaction tests, the compound was used by dissolving 1 tablet per liter of water which provided 1000 to 1,300 mg/1 of test solutions. The solution of the 8-HQS was poured over the contents of the compactor prior to compaction; approximately 1/2 liter of solution was used for each compaction cycle.

Because of the limited quantity of 8-HQS available, it was decided to run a preliminary screening test against typical mixed microbial flora isolated from the wasted food items and trash. The main purpose of these test was to determine the microbial load present in the wasted food and if the 8-HQS was effective against the microorganism typically found in the food waste when determined by the disc zone of inhibition test. The phenolic disinfectant was also evaluated at the same time for comparative purposes.

Test tubes containing five grams each of a homogeneous waste sample and various concentrations of disinfectant were incubated at room temperature for the plate counts. Each day for three days one test tube from each group, each with a different concentration of disinfectant, was used to make a series of serial dilutions in 0.8% saline. The dilutions were plated out using a spreader technique on plates of Letheen agar (see Table 1 for composition) and incubated for 24 hours at 35 C.

The zone of inhibition disc-test procedure involved the use of filter paper discs dipped in a solution of the disinfectant to be tested and air-dried. The discs were applied to nutrient agar plates seeded with a mixed inoculum obtained from bag #47 and grown for 20 hours at 35 C in nutrient broth. The seeded plates were then incubated for 24 hours at 35 C.

TABLE 1

COMPOSITION OF LETHEEN AGAR

	Grams
Armour peptone (peptonum siccum)	10
Difco beef extract	5
NaCl	5
Lecithin – (Clearate WDF)	0.7
Tween 80 (Sorbitan monooleate)	5
Bacteriological agar made up to 1000 mls with water should be 6.8 pH	15

NOTE: Azolectin is preferable to Clearate WDF.

RESULTS

It was determined that the KCB-1 bags used in the investigation could tolerate an average CO_2 pressure of 0.13 psi (0.0009 Pa) generated inside the bag after the additional heat seals were applied across the bottom and top of each bag. Table 2 summarizes the results of the dry ice tests used to determine the maximum amount of gas pressure the heat-sealed KCB-1 bags could withstand.

Table 3 tabulates the original and dimensional changes (ballooning) observed in the initial experiments with the KCB-1 bags. Bag #7 was the first to balloon. This was thought to be due to a cream of mushroom soup that had been included in the food wastes. Consequently, cream of mushroom soup was added to most compacted bags starting with bag #13. However, only one more bag of the next six bags showed ballooning. It was decided that the excessive handling of the bags incubated in Room DB019 (each bag was carried outside the chamber to be measured due to poor visibility within the chamber caused by clouds of steam), and possibly the high humidity in the tropical chamber was responsible for small leaks observed in some of the bags. To prevent excessive handling of the bags while measuring their circumference and also to eliminate any possible effects of incubation under high humidity, all compacted bags starting with bag #19 were stored in form D036, a dry incubator with a temperature range of 33 to 34 C. In the dry incubator the circumference of each bag could be easily measured without moving any of the bags. An immediate increase in the percentage of the bags showing ballooning was observed under these improved conditions as noted in Table 4 for bags #19 to 23. It was established that ballooning could be used as a criterion for effectiveness of the disinfectant, therefore starting with bag #25, disinfectant was added to most of the bags in an attempt to prevent ballooning. The results shown by bags #25 to 51 (see Tables 4 and 5) indicate that the phenolic disinfectant distributed by pouring a solution of it over the waste prior to compaction does not routinely prevent ballooning and odor production inside the bags at the levels of disinfectant tested. Nine of 14 bags treated with phenolic disinfectant in concentrations ranging from 1 oz. in two liters of water (=14,000 mg/1) to 4 oz. in one liter of water (using 1 1/2 liter solution = 168,000 mg/1) showed ballooning and both bags treated with 8-HQS did likewise. Two possible reasons for this, other than less than optimal concentrations, are that the disinfectant does not affect all microorganisms found in the trash, or, more likely, that the disinfectant is not reaching all the areas in the waste material.

Table 6 shows the type of bacterial counts obtained. In general, the phenolic disinfectant shows a reduction in the counts; however, there is an anomaly in the figures reported. The irregularities with numbers reported in Table 6 are due to the small particles of garbage that adhered to the sides of the test tubes and did not become saturated with the disinfectant despite thorough shaking. Consequently, the figures in Table 6 only represent a qualitative trend rather than absolute values.

TABLE 2

MAXIMUM CO_2 PRESSURE DEVELOPED WITHIN PLASTIC BAGS AT POINT OF BURSTING

Bag#	mm of Oil	mm of Hg[a]	psi[b]	Pa[c]
A	102	6.54	.13	0.0009
B	101	6.48	.13	0.0009
C	87	5.58	.11	0.0008
D	104	6.67	.13	0.0009
E.	114	7.31	.14	0.0009
F	102	6.54	.13	0.0009

[a]mm Hg = .06416 x mm oil

[b]psi = .01934 x mm Hg

[c]Pa = psi x .006895

TABLE 3

DETERMINATION OF BAG BALLOONING AS A MEASUREMENT OF MICROBIAL GAS PRODUCTION

Bag #	Days of Incubation: 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	Bag Circumference – Inches[1]																					
1	48	48			47½	47½	40	46R	48			46	45	46	46				45		46	
2	48	48			48	47	46	46	48			46	46½		46				44½		45	
3	43			44	44	42	43	42½			42	41		41				41		41		
4	44			44	43	42½	42R	42			41	39		40				39		39		38½
5	47½	45½	46½	47	47			46½	48		47½				47		47½		45½			
6	47	47½	47			47	46		47				46		47		46			44½		
7	48	49	49			49½	48½		48½				48									
8	50½	51	51½			51½	52		51½				52		52½							
9	42	43			42	42		42				42½		42		42			41½	41	39½	
10	46	47			46R	46		47				46½		47		46			45	44½	43	
11	50½			50½	50½																	
12	50½	50		51				51														
13	45		46				46		45½		44½			44	44	43½		43½			45	
14	47				45		45		44½			44½	45	43½		43			44			44½
15	42½				50		46		45			44½	45½	45½		44½			45			44½
16	41				43½		43		44½			43	42½	42		42			42			40½
17	45			47½		45		45½			44	43	43		42			43			42½	
18	43½		44		44½			43	45½	44		44			44½			43				

R = Bag reheat-sealed at this time.

Circle around measurement (50) indicates bag shows obvious ballooning. All measurements are in inches and represent the circumference taken over the top of the bag perpendicular to the heat seal.

Blank space indicates no measurement taken on that day.

A solid vertical line followed by a solid horizontal line ________ shows that the bag has been discarded.

TABLE 4

DETERMINATION OF BAG BALLOONING WITH AND WITHOUT DISINFECTANT

Days of Incubation *

Bag Circumference – Inches [1]

Bag	#	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	19	43	46	46½			46½	47	48	47	45½			45	43½	43½	44	43			40½		
	20	41	42½	41½			41	44½	43	43	41			41	41½	41	41	41			40		
	21	43	42½			45½	47	45½	44½	43½			43½	43	42	42½	43			42			
	22	42½	43			45	45	46½	43½	43			43	43	41½	42	42			41½			
	23	42			43½	44	43	42	43½			41½	42	43	42	41½			42½				
	24	42½	44½	44½	43	43			43	42	43	42											
P	25	44½	48½	45½	45½	45			45	44	44	44											
	26	44	44	44			46½	47	45½	44½	44½			43½	42	41	41½					40	
P	27	41	41	41			42	41	41½	41	40			41	39½	39	39½					38½	
	28	41			40	40	40	39½	39½			39	39	39	40					39½			
	29	40½			45½	45½	46½	46	45			41	40½	40½	40					40			
P	30	41½			43	42½	44	43	43			40	40	41	40					39½			
P	31	43½	44½	45	44½	44			41½	41	41½	41					40½						
	32	43½	44½	46			43	43	43	42													
P	33	42	41½	42			43	42½	42	41													
	34	42	41½			46½	46	44	44	43½				42	41½	41							
P	35	42			47½	47	46	45	45				43½	44½	44½								
	36	42	41½	42	41				41	41	41												
8HQS	37	41½	46	45	45½				43	43	41½												
	38	41	42	43½				41½	40	39½	39½												
P	39	42½	45	45				43	42½	41½	42												
8HQS	40	40	43½	43½				43½	43½	41½	41												
	41	40½				42	43	43	42½				40½										
	42	41				42½	41	41½	42				41½										

W = Wet spot observed under bag.
P = Phenol added to that bag.
8HQS = 8 Hydroxyquinoline sulfate added to that bag.
R = Bag re-heat-sealed at this time.
Circle around measurement (50) indicates bag shows obvious ballooning.
All measurements are in inches and represent the circumference taken over the top of the bag perpendicular to the heat seal.
Blank space indicates no measurement taken on that day.
A solid horizontal line ______ shows the bag has been discarded.
*To convert to cm, x by 2.540.

TABLE 5

DETERMINATION OF BAG BALLOONING WITH AND WITHOUT DISINFECTANT

Bag	#	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Odor at End of Test
		Days of Incubation — Bag Circumference — Inches[1]																				
	43	43½	47	46½	47				45	45½	44½	44			43½	42½						++
P	44	41½	45	48				45	45	45½	46½			41	41							+++
	45	38	36½	R			39½	43	44½	44½			44	40								+++
P	46	36½	41½				37½	38	40	39			38	37								+++
	47	36½				44	45	44½	44			41	41									++
P	48	34				39½	35½	35	34			33½	33	33								++
P	49	33½				40	38	35	33			30½										+
P	50	37½				43	41½	41½	41			38½	38	37	37	37			36½	36½	36½	++
P	51	43	42½	42	44			47	46½	45	45	44½			43	43	41					+
P*	52	34	35	35			39½	38½	39													+
P	53	39	39	40R			40	39½	39	40	39½			40	39½	39						++
P*	54	38½	39			39	39	37	37	36½			36½	37	35							++
P*	55	34			42	37	34½	34½	34½			32	32	32								--
P*	56	34	33½	32½	33½	35			37½	38	36½	36½										--
	57	39	38½	37	37	38			36½	37		36										++
P*	58	37½	37	35½	36			37	36½		36											++
P*	59	36	36	36½			36	35W		36												+
8HQS	60	30	30	30			30	29½		29												+
P*	61	34	33½			34	34		34													
	62	36			39½	39	37	34														
P*	63	35½			42½	40	40	39														
P*	64	37			41	40	39½	39														
P*	65**	36	36		37																	

P = Phenol added
P* = Soaked in Phenol
** = Incubated at 21°C
R = Bag re-heat-sealed at this time
Circle around measurement (50) indicates bag shows obvious ballooning.
All measurements are in inches and represent the circumference taken over the top of the bag perpendicular to the heat seal

[1] To convert to cm, x by 2.540.

Blank space indicates no measurement taken on that day
A solid vertical line followed by a long dash shows the bag has been discarded
Odor: -- no odor; - very slight odor, not disagreeable; + some odor, slightly disagreeable; ++ strong odor, disagreeable and usually acrid; +++ very strong acrid odor and very disagreeable

TABLE 6

ESTIMATE OF AEROBIC COUNT IN GARBAGE[1] BEFORE AND AFTER CONTACT WITH PHENOLIC DISINFECTANT

Disinfectant Concentraion (%) by Weight	Before Disinfectant Added-0 Hours	Counts[2] /gram of Garbage After			
		4 Hours	1 Day	2 Days	3 Days
0	4730	5250	71,660	>300,000	>300,000
1.40	3890	6720	14,930	5,350	>300,000
.70	2920	5460	27,070	45,100	30
-	4260	6180	28,950	28,300	4,170
	(3950 average)				

[1] The garbage sample consisted of:

barbecued pork	–	36% (by weight)
roast beef	–	36%
lettuce	–	5%
mayonnaise	–	4%
dry milk (liquid)	–	19%
Total		100%

[2] See explanation on p.19 for the poor bacterial reduction noted for sample treated with 1.40% disinfectant.

The zones of inhibition that were observed are listed in Table 7 and are shown in Figure 3. The phenolic disinfectant and 8-HQS are both effective inhibitors *in vitro* against microorganisms common to the trash. The two distinct zones of inhibition simply indicate two different degrees of susceptibility to the disinfectant.

It was thought that the organic matter found in food wastes might be inhibiting the activity of the disinfectant in the bags of compacted trash. Disc tests (see Table 7 and Figure 3) showed that a meat extract consisting of ham, pork, and sausage did not inhibit the disinfectant. Since other meats and vegetable material were not tested, it is therefore possible that other sources of organic matter could inactivate the disinfectant.

The most probable reason for gas and odor production in the disinfectant-treated bags is that the disinfectant was not being evenly dispersed so that it could make contact with the wastes harboring microorganisms. To rectify this, the majority of the bags starting with #52 were filled with trash and garbage that had been previously allowed to soak in the disinfectant for periods ranging from 20 minutes to 2 1/4 hours. Five out of nine bags of compacted trash treated in this manner also showed ballooning indicating that even soaking the food wastes and saturating the paper wastes does not eliminate all gas-producing microorganisms. (See Table 5).

Odors in these bags, however, were generally not as disagreeable as those produced in the untreated bags and those bags that were treated by pouring the disinfectant over the wastes before compacting. Bags up to #51 usually had a strong acrid odor of decay when checked after 10 to 20 days of incubation. The bags containing soaked food wastes varied between a strong, sour food odor to no disagreeable odor at all. Furthermore, when a definite odor was present, it was never an acrid odor, but rather an odor of some food waste that was in the trash, such as coffee grounds.

Since the lower concentrations of the disinfectant as recommended for use in latrine buckets (see specification O-D-1435A, Type II) failed to give adequate control, it was decided to increase the disinfectant application to a 5% level. This concentration was based on the Whirlpool studies (reference 1) which determined that the 5% concentration was satisfactory for holding fecal waste in an innocuous condition under aerospace conditions in both the Gemini and Apollo space flight missions.

The increase of the concentration of the phenolic disinfectant to 5% showed better control (bags #69 to 109, Table 8) since these bags, after 4 weeks incubation were generally rated as having some slight odor of phenol, fermenting or slightly spoiled. However, only two of the seven bags containing the 5% phenolic disinfectant were considered acceptable.

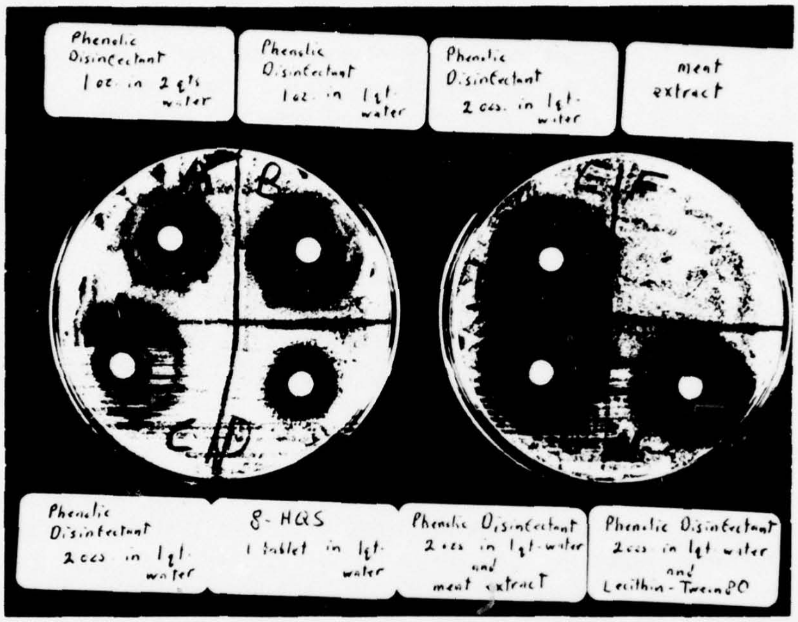

Figure 3. **Concentric zones of inhibition against mixed garbage inoculum obtained with paper discs soaked in various concentrations of phenolic and 8-hydroxyquinoline sulfate disinfectants: A ≅ 14,000 mg/l, B ≅ 28,000 mg/l, C ≅ 56,000 mg/l, D ≅ 1,300 mg/l, E ≅ 60,000 mg/l, F meat extract only, G ≅ 60,000 mg/l w/meat extract and H ≅ 60,000 mg/l w/.07% lecithin and 0.5% tween 80 inactivators.**

TABLE 7

AVERAGE ZONE[1] OF INHIBITION TEST RESULTS OBTAINED WITH SEVERAL CONCENTRATIONS OF THE PHENOLIC DISINFECTANT AND 8 HYDROXYQUINOLINE SULFATE

Plate No.	2% Meat Extract	~15,000 mg/l Phenolic Disinf.	~30,000 mg/l Phenolic Disinf.	~60,000 mg/l Phenolic Disinf.	~60,000 mg/l Phenolic Disinf. with 20% Meat Extract	~60,000 mg/l Phenolic Disinf. & .07% Lecithin & 0.5% Tween 80	~1,300 mg/l 8-Hydroxyquinaline Sulfate
1	None	9,26	12,28	15,33	15,33	12,29	11,20
2	None	9,26	14,30	15,34	15,34	14,31	10,20
3	None	8,18	9,24	12,28	14,31	13,30	uneven
4	None	9,23	9,25	14,31	15,33	14,32	11,20
5	None	9,23	12,30	13,28	14,32	12,31	17,17
6	None	9,28	12,30	15,32	16,33	13,31	15,15

[1] The two values reported in each column represent the diameter in mm of the inner and outer concentric zones as noted from each disc as seen in Figure 3.

TABLE 8

INHIBITORY EFFECT OF SEVERAL COMPOUNDS LAYERED ON OR MIXED IN GARBAGE UTILIZING THE FERMENTOR SCREENING PROCEDURE

	Days 0	1	2	3	4	5
			Ml_1 Gas Produced			
Control	0	600				
1.9% Salt***	0	485	600			
3.3% Salt***	0	40	100	115	125	130
4.9% Salt**	0	45	80	100	–	145
6.1 Salt**	0	80	150	–	–	–
6.5% Salt**	0.5	30	55	60	–	150
6.8% Salt**	0	1	10	10	10	10
8.2% Salt*	0	0	0	0	0	60
6.6% Calcium Chloride**	5	130	–	–	–	130
3.4% Propylene Glycol**	0	50	125	135	140	140
6.3% Propylene Glycol**	5	130	175	–	–	500
6.6% Prypylene Glycol**	10	350	–	–	–	350
3.3% Sodium Silicate**	0	65	300	>500	–	–
6.6% Sodium Silicate**	0.5	125	310	–	–	600

* Blended with a homogenous garbage.

** Added at interface of alternate layers of mashed potatoes, hamburger and mashed potatoes in 1 liter fermentor jar.

*** Layered between alternate layer of a homogenous garbage in 1 liter fermentor jar.

In an attempt to improve the performance of the phenolic disinfectant, several runs were made using a mixture of 2% sodium chloride and 5% phenolic disinfectant (bags #70 to 110, Table 8). Only one of the seven tests using this combination of disinfectant gave a + odor rating and it was described as alcoholic/phenolic, not putrefactive. Thus, the combination of salt and disinfectant appears to give good control against microbial breakdown of the compacted food waste.

Table 8 shows the inhibitory affect of several compounds when evaluated by the fermentor screening procedure when mixed or layered on garbage. Although the literature shows that most of the organisms can be controlled by 6% salt, these tests indicate some gas production can be produced even when 8.2% salt is mixed with a uniform garbage. Even though the preliminary screening experiments did not always give the control anticipated, it was decided to conduct the bag/compaction tests since the screening tests do not simulate the environment found in the bags which contain a mixture of food waste and inert trash.

Bag compaction experiments utilizing 2 to 10% salt (bags #68, 74, 79, 85, 95, 102, 108, 113, 121, 117, 122 and 123) are reported in Table 9. After 4 weeks incubation, 8 out of 8 heat sealed bags were odor rated from no odor (garbage odor only) to slightly decomposed. Two of these bags, however at the 2% salt level did show signs of gas production after 4 days (bag #121) and 7 days (bag #113). When an odor other than the normal garbage odor was present, it was rated as alcoholic, slight fermenting odor, aromatic, etc., while the control containing no preservative was rated as having a medium decomposed odor with gas production after 7 days incubation. The bags containing 4 to 8% salt (bags #121, 117, and 122) and residual food wastes from AF frozen meals were rated as +0, (very, very slight odor) with a slight fermentation odor; the bag containing 4% salt also produced some gas after 4 days incubation. At 10% salt (bag #123), only a garbage odor was noticed (0 – same as day 0). In the series of bags (bag #'s 130 to 139) containing 1 box of salt (1.63 lb = 880 g) per bag, salt concentrations ranged between 8.5 and 12.5%) depending on the weight of bag contents. All of these bags were rated as 0 while the control was rated +++ (heavy decomposed odor) and putrid.

Tables 8 and 9 show that experiments with sodium silicate at 3.3 and 6.6% in the fermentor tests and at 2 and 6% in compaction tests (bags #115 and 119) failed to provide the required odor and gas control and were therefore abandoned.

Because Aquacide III has dehydrating properties and is a non-aqueous form of ethyleneglycol, it was evaluated directly in the compacted bags (#'s 143, 144, and 145) at 8.6 to 9.4%. Table 10 shows that these concentrations of Aquacide III were totally ineffective in controlling putrefaction of the waste and was not further evaluated at higher levels because of its high price. Drierite, another dehydrating agent, was also evaluated (bag #127 at 4.5%) and likewise found to be totally ineffective at this concentration.

TABLE 9

OBSERVATIONS ON THE EFFECT OF VARIOUS MICROBIAL INHIBITORS IN CONTROLLING BALLOONING OF COMPACTED FOOD WASTE/TRASH IN HEAT SEALED BAGS

Bag #	Inhibitor	Day O Bag Measurement – inches	1	2	3	4	5	6	7	8	9	10	11	12	Objectionable Odor Rating End of 4th Week Incubation	Description of Odor
71	Control	42		48"B											+++	strong, cheesy
69	5% GPD MG	43													+	phenol
75	5% GPD MG	39													+	slight cheesy, phenolic
80	5% GPD MG	35													+	phenolic, slightly spoiled
86	5% GPD MG	38													+	phenolic fermenting
96	5% GPD MG	37													+	phenolic fermenting
103	5% GPD MG	38													+0	phenolic
109	5% GPD MG	38													+0	phenolic
78	Control MG	39								46"B					+++	ammonical spoiled
77	1300 mg/l HQS MG	40													+0	aromatic
82	1300 mg/l HQS MG	37													?	strong HQS odor
88	1300 mg/l HQS MG	37													+	alcoholic, spoiled
112	Control MG+g	39							47"B(Deflated 2 weeks)						++	heavy cheesy odor, pungent
68	2% NaCL MG	42													+0	aromatic (lettuce)
74	2% NaCL MG	38													0	coffee grinds
79	2% NaCL MG	36													0	no odor
85	2% NaCL MG	38													+	alcoholic
95	2% NaCL MG	39													+0	slight fermenting odor
02	2% NaCL MG	38													+	slight fermenting odor
.08	2% NaCL MG+g	39													+0	slight fermenting odor
113	2% NaCL MG+g	38							42"B(Deflated 1.5 weeks)						+	strong fermented odor

TABLE 9 (cont'd)

OBSERVATIONS ON THE EFFECT OF VARIOUS MICROBIAL INHIBITORS IN CONTROLLING BALLOONING OF COMPACTED FOOD WASTE/TRASH IN HEAT SEALED BAGS

Bag #	Inhibitor	Day O Bag Measurement – inches	1	2	3	4	5	6	7	8	9	10	11	12	Objectionable Odor Rating End of 4th Week Incubation	Description of Odor
121	4% NaCl MG+g	39				5"B									+0	slight fermented odor
117	6% NaCl MG+g	38													+0	slight fermented odor
122	8% NaCl MG+g	40													+0	slight fermented odor
123	10% NaCl MG+g	37													0	faint food odor
70	2% NaCl + 5% GPD MG	43													+0	phenolic
76	2% NaCl + 5% GPD MG	39													0	phenolic
81	2% NaCl + 5% GPD MG	38													+0	food odor
87	2% NaCl + 5% GPD MG	38													+	alcoholic, phenolic*
97	2% NaCl + 5% GPD MG	40													0	no odor
104	2% NaCl + 5% GPD MG	38													+0	phenolic
110	2% NaCl + 5% GPD MG+g	39						41"SB(Deflated 1.5 weeks)							0	phenolic
105	2¼ lbs. NaCL MG	41													+0	slight fermented
111	1½ lbs. NaCL MG+g	39													+0	slight fermented
114	2% Prop. Glycol MG+g	39													+	strong fermented odor
118	6% Prop. Glycol MG+g	38						43"SB(Deflated 1.5 weeks)							+0	strong odor
137	26% Prop. Glycol FD	–													0	strong odor
115	2% Na Silicate	39													++	strong, pugent, cheesy
119	6% Na Silicate	39													+++	putrid

MG+g = mixed garbage/trash with 1% glucose in mashed potatoes
MG = mixed garbage
SB = semi-balloned
FD = frozen dinners

TABLE 10

OBSERVATIONS ON THE EFFECT OF VARIOUS MICROBIAL INHIBITORS IN
CONTROLLING ODOR/GAS PRODUCTION OF COMPACTED FOOD WASTE/TRASH IN NON–HEAT SEALED BAGS

Bag #	Inhibitor	Contents	Days of Odor Control 1	2	3	4	5	6	7	8	9	10	11	12	13	14	Odor Rating, End of 4th week incubation
89	Control	FTARC							++								+++
91	5% GPD	FTARC				+			++								++ slightly decomposed
106	5% GPD	FTARC															0 garbage odor
92	1 lb NaCL	FTARC							0								0 garbage odor
100	1½ lb NaCL	MG		0				0									0
98	2 lbs NaCL	MG		0				0									0
124	1 lb NaCL (4%)	MG		0	0			0	0								+ slight odor
126	*Control*	FD	0			0			+				+				+++ putrid
125	1lb Control (4%)	FD	0	0	0			0	0						0		+ slight odor
128	1.63 lb Control (6.5%)	FD						0	0								0
129	Control	FD							+							+++	+++ putrid
130	1.63 lb NaCL (9.5%)	FD							0							0	0
131	1.63 lb NaCL (9.3%)	FD							0							0	0
132	1.63 lb NaCL (8.5%)	FD							0							0	0 coffee odor
133	1.63 lb NaCL (9.0%)	FD							0							0	0
134	1.63 lb NaCL (9.3%)	FD							0							0	0
135	1.63 lb NaCL (9.3%)	FD							0							0	0 coffee odor
139	1.63 lb NaCL (12.5%)	FD							0							0	0
127	4.5 Drierite	MG	0			0			+							+	+++ Putrid
138	9.5% Hyamine 1622	FD							0							0	+0 musty
140	8.6% Hyamine 1622	FD							0							0	0 strong coffee odor
141	8.4% Hyamine 1622	FD							0							0	0 very strong odor
142	8.6% Hyamine 1622	FD							0							0	0 coffee/alcohol odor
143	8.6% Aquacide III	FD							+0							++	+++ obnoxious
144	8.8% Aquacide III	FD							++							++	+++ putrid
145	9.4% Aquacide III	FD							+0							++	+++ putrid

FD = Frozen dinners

FTARC = Flip top aluminum ration cans

MG = Mixed garbage

Hyamine 1622 at concentrations ranging between 8.4 and 9.5% as seen in Table 10 shows good control over the odor production in bags #140, 141, and 142. The only odor observed in these bags was that of the original food waste itself which maintained a strong coffee odor; bag #138 at the 9.5% level, however, did permit a musty odor to develop after 4 weeks incubation. The only explanation for this latter anomaly is that the Hyamine did not get sufficiently dispersed throughout the trash.

DISCUSSION

These studies emphasize a series of limitations and problem areas inherent in the concept of compacting garbage with trash. For example, the Kitchen Aid Compactor is not designed as a substitute for a food waste disposer and its use without modification for compacting both trash and garbage as outlined in the AF 4–12 Requirement is contrary to the manufacturer's design and recommendations. Thus, one objective of these studies was to determine if the Kitchen Aid Compactor can be so modified or adapted to overcome inherent design limitations, so that it will meet the AF requirements previously stated. Some of these limitations and possible solutions are discussed below.

The reported studies indicate that the original concept of manually adding a disinfectant to the trash/food waste mixture or soaking the trash/food waste in the disinfectant prior to compaction will not always guarantee complete control over odor and gas production. Tests showed that neither the phenolic disinfectant, nor the 8-HQS can physically wet out all the particles of food waste encountered and therefore do not permit the intimate contact necessary between the disinfectant and the waste food to insure the reliable degree of microbial inhibition required. The only probable way an additive disinfectant could be guaranteed to be effective in this system is to have the trash/food waste ground into a homogeneous mass with a disinfectant and a wetting agent, and thereby hopefully permit better distribution and surface contact between the trash/food waste mixture and the disinfectant prior to and after compaction. Such a device would, however, require additional space and power aboard the aircraft, and for these reasons was not evaluated in the current study.

Hyamine 1622 is an example of a combined disinfectant-wetting agent. The Hyamine performed well without mixing in three experiments (bags #138, 140 and 141, Table 10) probably due to its dual nature of being both a disinfectant and a wetting agent. The Hyamine had to be absorbed onto sodium sulfate to provide a solid product, and because of this it exerts about the same corrosive properties as does sodium chloride since it too is classified as a neutral salt. Hyamine can also be absorbed onto at least eight other salts in various proportions including powdered borax. Absorption onto borax could conceivably enhance the inhibitory properties of Hyamine since borax (sodium borate) is, itself, a feeble antiseptic and used commercially in curing and preserving of hides. One disadvantage noted when the Hyamine was absorbed onto sodium sulfate was that the final product produced fine particles which would tend to float in the air in the form of a fine dust. In adding this compound to the compacted food waste, it would cause the laboratory technician to sneeze, thus indicating it would not be desirable aboard aircraft unless it could be prepared in a nondusting form. Its producer (Rohm and Haas) also cautions that it can cause skin irritation or damage to the eyes and contamination of food should be avoided.

The 8-HQS disinfectant was not fully evaluated in these studies for the reasons previously mentioned. However, it is noted in a recent Russian article[11] that food wastes from a dinner table were all well preserved by a 1:1 mixture of quinosol (8-hydroxyquinoline sulfate) and salicylic acid, in that spoilage microflora were suppressed while fermentation flora were unaffected. The paper states this combination might be useful in preventing decay odor from food wastes on spacecraft. It would appear that the 8-HQS alone is not sufficiently effective to prevent microbial spoilage in food wastes, and that the addition of salicylic acid is required to provide the essential control over the odor producing micoorganisms. Although we have not conducted any tests to confirm this finding, the literature tends to indicate that 8-HQS has certain deficiencies; namely, it is not an effective inhibitor against some bacteria. This is further substantiated by the Russian researchers when they reported that the fermentive flora were unaffected by the addition of the mixture of the two biocides. It is likely that the Russians were also experiencing similar difficulty in obtaining an intimate mix of garbage and preservative as indicated by the ineffective control over the fermentative bacteria in their experiments.

The most effective and most inexpensive preservative evaluated in these studies was salt. The action of salt is not a disinfectant by the usual criteria which involves a physiological poison route. The action of salt as a preservative is usually considered to be due to plasmolysis. Plasmolysis (the exit of water from microbial cells when placed in a solution which has an osmotic pressure higher than the cell constituents and which will continue until a balance or equilibrium is established between the osmotic pressure within the cell and the surrounding medium) is a physical process which exerts a marked bacteriostatic action and has long been utilized in the preservation of foods. Jellies, candies, candied fruits, salted meats and fish of all kinds are examples where foods are partially protected from microbial decomposition by plasmolysis caused by sugar or salt. Although plasmolysis undoubtedly plays a major role in the inhibitory activity of salt, its action in preserving food or preventing microbial spoilage is not fully understood since it does not appear to act solely by virtue of its osmotic effect. Spiegelberg[12] showed that the osmotic pressure at which growth of bacteria ceases is much lower for salts than for sugars. The concentration of salt necessary to inhibit the growth of microorganisms in food is related to many factors, including pH, temperature, protein content and the presence of inhibitory substances such as acids. The water content is obviously a major factor also

[11] Rogatina, L.N., L.T. Poddubnaya, N.A. Kamennov, I.G. Popov and P.P. Lobzin. 1975. Conservation of Food Residues with a Mixture of Quinosol and Salicylic Acid. Kosm. Biol. Aviakosmichiskaya Med. **9**: 44-48.

[12] Spiegelberg, C.A. 1944. Sugar and Salt Tolerance of *Clostridium pasteurianum* and some Related Anaerobes. J. Bact. **48**: 13-30.

since it is the concentration of salt in the water phase and not the amount in the food as a whole which is significant. Labrie and Gibbons[13] found that the inhibitory action of salt on bacteria increases with decreasing temperature from 21° to 10°C. Callow[14] also noted that the amount of salt required to inhibit mold growth decreased with decreasing temperature; 8 percent was required at 0°C whereas 12 percent was necessary at room temperature. In addition, the composition of the medium in which the organisms are growing has been repeatedly demonstrated to influence the degree of salt tolerance demonstrated by microorganisms. Garrard and Lockhead[15] observed that organisms exhibited greater salt-tolerance in curing pickle than in broths of similar salt content. Stuart[16] showed that growth of halophilic bacteria may be either stimulated or retarded by varying the protein content of the medium. The effect of pH on salt tolerance was studied by Joslyn and Cruess[17] who found that lowering the pH caused a marked decrease in the salt tolerance of various yeast and mold species.

Microorganisms have been divided into halophiles and non-halophiles based upon their response to salt concentration. This demarcation may be misleading since many intermediate types exist and there are reports for example, of obligate halophiles growing on low salt media. Contrary to the general belief that halophilic bacteria are exclusively associated with a salt environment such as sea water and fish, Stuart[18] found that they are widely distributed in nature and can be isolated in 25 percent salt media from non-salt environments such as stagnant water, sulfur springs, rat-dung and soil, provided an incubation period up to 90 days is permitted. From a practical application, salt appears to offer good inhibition potential against most of the microorganisms food waste might contain, and those microorganisms which require extended incubation would not appear

[13] Labrie, A. and N.E. Gibbons. 1937. Studies on Salt Fish II. The Effect of Salt Concentration on Preservation. J. Biol. Bd. Canada **3**: 439-449.

[14] Callow, E.H. 1929. Food Invn. Bd. Ann. Rept. p. 72, HMSO, London.

[15] Garrard, E.H. and A.G. Lockhead. 1939. A Study of Bacteria Contaminating Sides of Wiltshire Bacon with Special Consideration of their Behaviour in Concentrated Salt Solutions. Canad. J. Res., Sect. D, **17**: 45-58.

[16] Stuart, L.L. 1940. Effect of Protein Concentration and Cysteine on Growth of Halophilic Bacteria. J. Agr. Res. **61**: 267-275.

[17] Josyln, M.A. and W.V. Cruess. 1929. A Comparative Investigation of Certain Film — Forming Fungi. Hilgardia **4**: 201-240.

[18] Stuart, L.S. 1938. Isolation of Halophilic Bacteria from Soil, Water and Dung. Food Res **3**: 417-420.

to be a problem since the original criteria specified in the AF requirement stated that the inhibitor would only be required to be effective for a period of up to 30 days.

The main objection to the use of salt would appear to be associated with potential corrosion of metals. Salts are classified according to their reaction when dissolved in water. Some salts from neutral or neutral-oxidizing solutions, whereas others hydrolyze to form acid, acid-oxidizing, alkaline, or alkaline-oxidizing solutions. Fortunately sodium chloride, potassium chloride, sodium, potassium and calcium sulfates are classified as neutral salts and fall into the category of being not as corrosive as the acid, acid-oxidizing and alkaline-oxidizing salts. Most salts are good electrolytes and they frequently induce galvanic corrosion. The decision as to whether salt can be utilized aboard the aircraft in question is a determination beyond the scope of this study. Whatever corrosive activity sodium chloride possesses might be compensated for by the positive characteristics it provides as a microbial inhibitor, its anticipated temporary or intermittent use and its low cost. It is without question that granulated salt is the most inexpensive and readily available inhibitor costing currently only about $0.08/lb in 2-lb. (907 g) bags. The 26-oz (780 g) boxes of salt cost 15 cents/box. A 100-lb drum of salt occupies an area 29 inches high by 14 inches in diameter, and at the rate of 26-oz of salt/day, the 100-lb drum would be sufficient for approximately 60 days of use. In addition, salt is odorless and has no toxicity or vapor pressure problems associated with it. Salt is essentially 99+% pure and therefore completely active. It is solid in form, will not freeze and has an indefinite storage life when kept dry.

Propylene glycol and flaked polyethyleneglycol were evaluated as possible substitutes because of the corrosive nature of salt. Unfortunately, the high concentration required, the liquid nature of propylene glycol and the ineffectiveness of polyethyleneglycol, as well as their costs, are adequate reasons for their rejection from further consideration.

RECOMMENDATIONS

As previously stated, the difficulty in obtaining the intimate mix between an added disinfectant and the garbage/trash mixture dictates against the use of a disinfectant unless mechanical mixing and the addition of a wetting agent are incorporated as part of the procedure. Since these operations would increase the cost in providing the degree of microbial protection required without any guarantee that a successful disinfectant/garbage/trash mixture can be practically achieved, it is recommended that salt treatment should first be field-tested to determine if it meets the AF criteria in actual use tests aboard aircraft and in missile sites. The attached Appendix will provide the necessary illustrated instructions on the use of salt as an inhibitor to be added to the trash/garbage mixture prior to compaction in a Kitchen Aid Compactor. If the total accumulation of food waste and trash amounts to 15 lb/day, the use of one 26 oz (780 g) box of salt would be at a 10.6% concentration; if the waste amounted to 20 lb/day, the salt concentration would calculate to 8.07%.

If the field tests with the salt inhibitor do not meet the AF criteria, additional tests can be undertaken by the Natick Research and Development Command to provide optional microbial inhibitors, but at a greatly increased cost since there is no other compound that can currently compete with salt for its low cost, availability, concentrated effectiveness and low toxicity.

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17. Joslyn, M. A. and W. V. Cruess. 1929. A Comparative Investigation of Certain Film-forming Fungi. Hilgardia. **4**: 201-240.

18. Stuart, L. S. 1938. Isolation of Halophilic Bacteria from Soil, Water and Dung. Food Res. **3**: 417-420.

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APPENDIX

APPENDIX

DIRECTIONS FOR USING KITCHENAID TRASH COMPACTOR WITH BAGS

NOTE: Compacting folded paper towels, napkins, newspaper etc on top of loose trash/waste food in the drawer will help keep the ram clean.

Steps 1-6 are identical to instructions received with the Use and Care Guide furnished with the Kitchenaid Compactor:

1. Lift Built-in Storage Compartment cover and leave in up position.

2. Pull Basket Release Handle down.

3. Hold folded bag by top and place inside Tilt-Away Trash Basket.

4. Open bag. Pull top edges of bag down over top of drawer.

5. Lock bag in place by pulling four outer holes of bag down over buttons on sides of drawer. Holes will expand over buttons without damaging bag.

6. Spread bag to fit interior of Basket. Close Storage Compartment cover. Pull Basket Release Handle up.

Steps 7 to 19 explain the use of salt as inhibitor when placing trash and wasted food together for compaction.

7. Place several thicknesses of paper (towels, newspapers, paper towels etc) in bottom of plastic bag lining the Basket. These papers will help hold any liquids that may be discarded. See Fig. 4.

8. Scatter or sprinkle approximately 3 to 4 tablespoons of salt on top of the paper in the bottom of the Basket before adding trash. Every effort should be made to distribute the salt evenly over the bottom layers of paper. See Fig. 5. (crystalline, orange colored potassium dichromate was used in place of salt for better *Photographic* contrast.)

9. Each disposable food tray should be generously sprinkled with salt at the completion of each meal. Assuming the average consumer will leave behind approximately 20% waste food, sprinkle approximately 1 tablespoon of salt across the surface of each disposable food tray prior to placing in the compactor rightside up. Each disposable cup should be sprinkled with about 1/2 teaspoon of salt; if there is still some residual beverage in the cup, place the salt in the bottom of the cup and let it dissolve in the beverage prior to placing right-side up in the compactor. Cutlery and other sharp objects should be placed toward the center of the load. See Fig. 6.

Figure 4. Step 7. Several thicknesses of newspaper, towels, napkins etc are placed in the bottom of the plastic bag to soak up any liquid that may be discarded with the food waste.

Figure 5 Step 8. Sprinkle 3-4-tablespoons of salt on the top of the paper placed in the bottom of the plastic bag before adding trash and food waste.

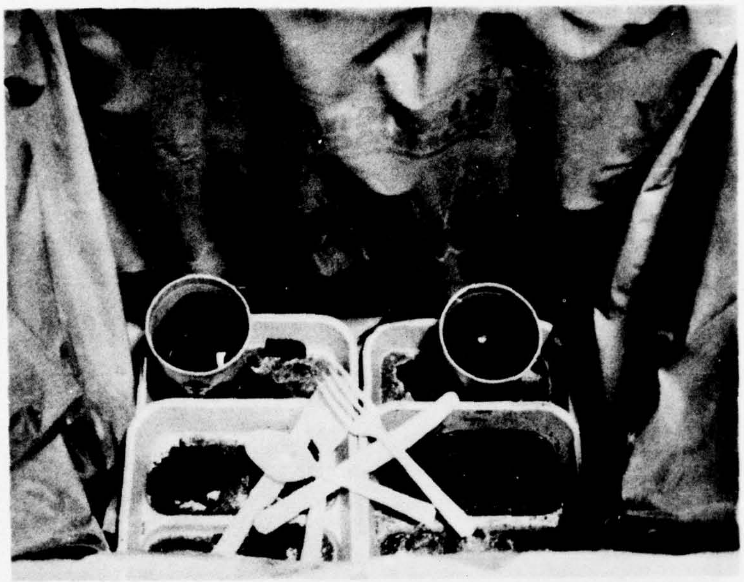

Figure 6. Step 9. Each disposable food tray should be generously sprinkled with about 1 tablespoon of salt at the completion of each meal. Each disposable cup should also contain about 1/2 teaspoon of salt by placing the salt in the bottom of the cup. The trays, cups and eating utensils should be placed in the bottom of the plastic bag in the compactor as shown.

10. After placing the salted used food trays, utensils etc into the compactor rightside up, place several thickness of paper (towels, newspapers, napkins, etc) over the last layer of food trays/trash which will help keep the ram in the compactor clean and prevents trash from sticking to it. See Fig. 7.

11. After each meal or when the compactor is full of loose trash/food waste, a compaction cycle should be run in accordance with directions found on page 8 of operating manual.

12. After the initial compaction, the top layer of paper and compacted material must be generously sprinkled with 3 to 4 tablespoons of salt as shown in Fig. 8. as was the first layer of paper placed in the bottom of the plastic bag. Prior to adding any additional used food trays, coffee cups, etc., each must be generously sprinkled with salt as described in paragraph 9 above. Again, before the next compaction, the top of the discarded trays/food waste must be covered with several thicknesses of paper, cardboard, napkins, etc., prior to compaction. Repeat the process as often as required.

13. The quantity trash/food waste resulting from three meals (15 man-crew/meal) plus snacks, etc are readily accommodated in the compactor for each day before it is necessary to remove the compacted trash. The partially filled bag may be removed after the last meal and final compaction, or removed when trash is compacted to about four to five inches from the top of the Basket.

14. Prior to removing the compacted trash from the Basket, sprinkle the last (top) layer of paper with about 3 to 4 tablespoons of salt as shown in Fig. 9. (Dark cardboard was used in the photograph for contrast and actual salt was used instead of potassium dichromate). Even distribution of the salt can be readily seen across approximately 2/3's of the surface of the last layer of cardboard.

NOTE: The bag should be removed after the last meal of the day or when trash is about four to five inches from the top of the Basket, whichever comes first. Steps 15 to 19 outline the changes in the procedure for the removal of the bag as outlined on p. 7 of Use and Care Guide[19] instructions.

15. Pull Basket Release Handle down.

16. Lift cover of Storage Compartment. Release bag from buttons. Pull up top edges of bag.

[19] Form No. 7478-B, "How to Get the Most of Your New Kitchenaid Trash Compactor; Use and Care Guide", The Hobart Manufacturing Co., Troy, OH 45374.

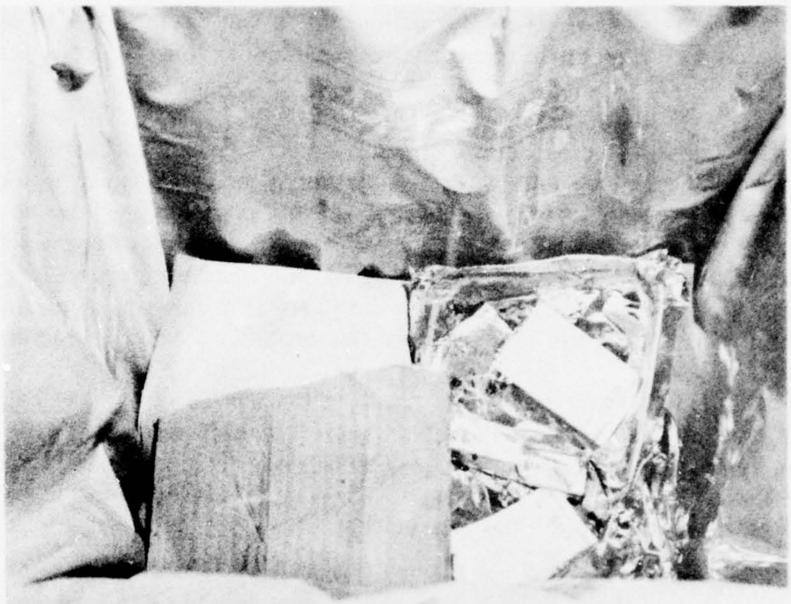

Figure 7. Step 10. After placing the trays, cups, eating utensils in the bag, cover them with a layer of paper towels, cardboard, napkins etc prior to compaction. This layer of paper will help keep the compacting ram clean.

Figure 8. Step 12. After the compaction cycle, apply 3-4 tablespoons of salt evenly across the layer of paper so that the compactor is ready to receive the trash and food residue from the next meal.

Figure 9. Step 14. At the end of the day when all food waste and trash have been compacted or when the compacted trash is about four inches from the top of the Basket, sprinkle the top layer of paper, cardboard, napkins etc with 3-4 tablespoons of salt.

17. Close bag securely by first twisting the free upper/neck portion of the bag several times 360° to the right and complete closure of bag with one of the twist ties provided.

18. Lift bag out of Basket and carefully inspect the integrity of the bag of compacted trash prior to placing it into second KCB-1 Bag. If the compacted bag shows rips or tears caused by sharp objects, these objects must be forced into the compacted bag with a flat object or removed so that they will not cause tearing of the outer bag. Place the bag of compacted trash in a second outer KCB-1 Bag as shown in Fig. 10 and seal the outer bag by first twisting the upper neck portion of the outer bag several times 360° to the right and complete closure of bag with one of the twist ties provided as shown in Fig. 11.

19. Store bags in area specified. Each day inspect stored bags for signs of ballooning caused by possible microbial gas production and odor. If a problem is noted, the cause is due to insufficient use of salt or its poor distribution throughout the trash/food waste mixture. To remedy, remove compacted trash from problem bag and work the compacted contents gradually into a new bag of non-compacted material with **1 cup of extra salt** over and above the amount normally used as described above. For example, if a problem bag is discovered and the used food trays/trash are about to be compacted, proceed as described above except only add about five salted cups and trays (or 1/3 of the trash to be compacted) and overlay with about 1/3 of the compacted trash and sprinkle with 1 cup of extra salt. Continue in alternate layers until the final 3rd layer of compacted material is placed on top and sprinkled with 1 cup of extra salt. If there is a residual of fluid in the bottom of the problem bag, add 1 cup of salt to the bottom of the bag being certain to distribute the salt as evenly as possible, fold bag and place on top of the compacted material along with a layer of paper, cups, napkins, etc., and compact in the usual manner. Tie bag off as previously described and continue to observe for any additional ballooning.

Figure 10. Step 18. Lift sealed bag out of the Basket and inspect for tears or rips. Place the bag of compacted trash in a second outer KCB-1 bag and close by twisting the upper portion of the outer bag several times 360° and complete closure with one of the twist ties provided.

Figure 11. Step 17. Prior to removing bag from compactor, twist the upper neck of the bag several times 360° to the right complete closure with one of the twist ties provided.